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DETERMINATION OF A JET FUEL METAL DEACTIVATOR BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

Paul C. Hayes, Jr.
Fuels Branch
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June 1983

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This technical report describes an analytical method that qualitatively identifies and quantitatively determines a metal deactivator additive used in Air Force JP-4 jet fuel. Relative retention time calculations, together with ultraviolet absorbance ratios at various wavelengths, were highly specific in assigning the tentative identification of the additive. A recovery standard that doubled as the internal reference standard for relative retention time measurements was crucial in correcting the found metal deactivator ppm level for losses

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in sample transfer, sample concentration, dilution, etc. A precision statement, reflecting the quantitative reliability of the method, cited +/- 0.5 ppm as the 95% confidence intervals for the true value of the predicted amount from the calibration curve for the metal deactivator in the 20-150 ppm range, after a 20-fold fuel concentration step. This crucial concentration step was necessary since the metal deactivator was at a level specified not to exceed 5.8 mg/liter, i.e. 5.8 parts per million (weight/volume) in JP-4 fuel.

FOREWORD

This technical report describes work performed under the In-House Work Unit 30480591 administered by the Fuels Branch (POSF), Fuels and Lubrication Division (POS), Aero Propulsion Laboratory (AFWAL/PO), Air Force Wright Aeronautical Laboratories. Project scientist for this program was Mr. Paul C. Hayes, Jr., who prepared this report.

Particular gratitude is extended to several individuals whose contributions ensured the success of this research effort. Teresa A. Boos (AFWAL/POSF) operated the liquid chromatograph, took the absorbance readings, and generated the calibration curves. Dwayne A. Burns (AFWAL/POSF) prepared the calibration solutions as well as the JP-4 fuels mixtures. The Quality Assurance Laboratory, Det. #13, SA-ALC/SFTLA, W-PAFB, Ohio, aided in evaporating the fuel samples. Mr. Edward C. Eimutis, a statistician with Monsanto Research Corporation (Dayton Laboratory), presented stimulating lectures and assistance in deriving the precision statements of all of the analyses contained in this report.

This report also represents the partial fulfillment of the requirements for a master's thesis by Mr. Hayes at Xavier University, Cincinnati, Ohio. The author wishes to express his deep appreciation for the guidance and encouragement offered by Dr. Joseph J. Klingenberg, the research advisor at Xavier University.

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LIST OF ABBREVIATIONS AND SYMBOLS

ABBREVIATION/SYMBOL	
HPLC	High Performance Liquid Chromatography
JP-4	jet propulsion fuel, wide-boiling range, conforming to MIL-T-5624L
MDA	metal deactivator, i.e. N,N'-DISALICYLIDENE-1,2-PROPANE DIAMINE
ACE	internal standard, i.e. ACETANILIDE
ppm	parts per million
mg	milligrams
µl	microliters
ml	milliliters
nm or λ	nanometers
µm	microns
mm	millimeters
cm	centimeters
min	minutes
n'	column's number of theoretical plates
t_R	component's absolute retention time
$t_{R'}$	component's adjusted retention time (t_R minus t_R of toluene)
RRT	component's relative retention time
$W_{0.5h}$	width of component's peak measured at half the peak's height in time units

SECTION I

INTRODUCTION

This study utilized High Performance Liquid Chromatography (HPLC) for the determination of N,N'disalicylidene-1,2-propane diamine (MDA), a specific additive used by the United States Air Force in turbine fuels. The end goal was to devise an analytical scheme for the qualitative identification and quantitative determination of this metal deactivator at a level specified not to exceed 5.8 mg/liter, i.e. 5.8 parts per million (weight/volume) in JP-4 jet fuel. JP-4 is a wide-cut distillate jet propulsion fuel conforming to Air Force Military Specification MIL-T-5624L. The fuel may have one of two metal deactivators, MDA and N,N'-disalicylidene-1,2-cyclohexane diamine (MDA'), but MDA is the more widely used. However, no analytical method exists for directly determining MDA or MDA' in jet fuels.

The function of a metal deactivator (MDA, Figure 1) is to convert trace amounts of divalent metal ions such as copper (II), lead (II), tin (II), etc. originally present in the fuel into inactive, soluble complexes. Uncomplexed metal ions can catalyze polymerizations in the fuel to yield gums which can deposit in and foul fuel heat exchangers and fuel filters. The rate of these reactions is directly proportional to temperature. A fuel with uncomplexed metal ions will consequently be less thermally stable than an identical fuel having its trace divalent metal ions complexed (Reference 1).

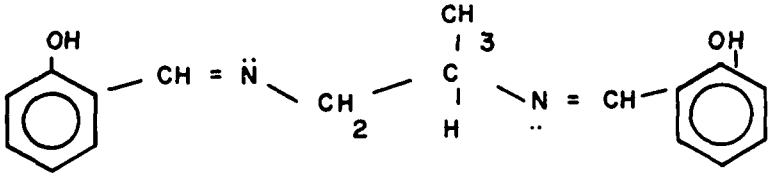


Figure 1. Metal Deactivator (MDA): N,N'-DISALICYLIDENE-1,2-PROPANE DIAMINE

The analytical scheme as developed in this report will:

a) qualitatively identify the MDA in a preconcentrated fuel sample by comparison of ultra-violet absorption data and relative retention time data;

b) and quantitatively determine the MDA by using appropriate calibration curves and a recovery standard.

HPLC was the analytical method of choice because of its high sensitivity, good resolution, and speed, as well as its ready adaptability to automation. Limiting factors in its use are the high equipment costs and the considerable experience needed to achieve reliable results.

SECTION II

HISTORICAL

Since the analytical method of choice to identify and quantitate the metal deactivator in JP-4 jet fuel was High Performance Liquid Chromatography, a short digression into the nature of this technique and its emergence to the forefront of analytical chemistry is warranted. Tswett first recognized and interpreted the chromatographic process based on his experiments using a chalk column to separate pigments in green leaves. The term "chromatography" was coined by Tswett to describe the colored zones that moved down the white column, i.e. "colored writing." Within the last decade, suitable high pressure pumps, efficient column packings, and sensitive low dead volume detectors have catapulted HPLC into a rapidly maturing and valuable complement to gas chromatography.

Chromatography is a wide-encompassing term describing a variety of separation techniques wherein a solute partitions between a moving phase, which can be a gas or liquid; and a stationary phase, which may be either a liquid or a solid. The form of chromatography used in this research was partition chromatography. The solute partitions between two immiscible solvents, one of which is fixed, i.e. stationary, and the other is the mobile phase. As the demand increased for more durable columns, packings with the stationary phase chemically bonded to the inert support were developed. This form of partition chromatography is called bonded phase chromatography. The commercially available column utilized in this study was of the bonded phase type with an aliphatic amine chemically bonded to the silica support. Packings based on silylation reactions have prevailed since the -Si-O-Si (siloxane) bond is very stable in most chromatographic solvents. These packings are generally stable in the pH range 2-7.5 and temperatures as high as $70^{o}C$ have been useable. When the stationary phase is more polar than the mobile phase, as in this research project, the chromatographic technique is called normal bonded phase chromatography (Reference 2).

Bonded phase packings offer distinct advantages over other packings:

a. Irreversible retention is less likely, since the very polar silanol groups (-Si-OH) have been converted to less polar linkages (-Si-O-Si-R);

b. No water for silica deactivation is necessary;

c. Solvent equilibration is rapid;

d. The packing materials are compatible with a wide assortment of HPLC solvents;

e. Selectivities are different for different polymeric phases.

Typical solvents used as mobile phases are mixtures of hydrocarbons, methylene chloride, tetrahydrofuran, etc. In these nonaqueous phases, a few percent of acid, e.g. acetic or phosphoric, may be added to suppress ionization and tailing of polar compounds, without damaging the column.

The heart of the chromatographic system is the column. It contains a stationary phase which in the case of this study was chemically bonded to a solid inert material called the support. The sample to be analyzed was injected into the column and moved across or through the stationary phase, being pushed along by the liquid mobile phase. Oftentimes, several atmospheres of pressure are required to elute the entire sample in a reasonable time. This is why HPLC is also called High Pressure Liquid Chromatography. In the form of liquid chromatography utilized in this research study, the various components in the injected sample were separated according to their degree of solubility in the stationary phase. Simply, it is a case of "like dissolves like." For a polar bonded phase column, polar compounds such as the metal deactivator are held or retained in the polar stationary phase longer than the relatively less polar aromatic hydrocarbons in the fuel matrix. Thus the MDA is

separated from the relatively less polar aromatics, and for that matter, the even less polar saturated and olefinic hydrocarbons. The fuel hydrocarbon matrix was hardly delayed at all; essentially, it did not notice the presence of the stationary phase and eluted from the column at almost the speed of the mobile liquid phase. The more polar MDA was retained considerably longer. When the MDA finally did elute from the column, it was identified and measured quantitatively by the variable wavelength ultraviolet detector.

SECTION III

CURRENT RESEARCH

1. EXPERIMENTAL

a. Instrumentation

The basic analytical instrumentation utilized in this study was the Varian Model 4200 Liquid Chromatograph equipped with a Varian VARISCAN Model 635 variable wavelength ultraviolet detector with flow cells designed for liquid chromatography. All injections were performed automatically with a Varian 8000 Autosample. A Spectra-Physics Model Auto-Lab System IV Computing Integrator initiated the injection sequence and collected the data with the calculations printed in report format on a Teletype Corporation "Teleprinter" Model ASR-33.

b. Liquid Chromatographic Conditions

A Whatman Partisil 10 PAC was used in this work and exhibited the following specifications: particle size of 10.0 μm, column length of 25 cm, column internal diameter of 4.6 mm, and a stationary liquid phase of a polymeric aliphatic amine bonded to silica gel. The optimized mobile phase composition (by volume) for this method was 97% methylene chloride and 3% glacial acetic acid. These solvents were the "distilled-in-glass" grade (Burdick and Jackson, Inc.) and used as is. The flow rate of the mobile phase was 1.0 ml/min, requiring a back pressure through the column of 350 psig. Other HPLC operating parameters were as follows:

(1) Temperature of the column was maintained at 25^{o}C via a water jacket through which distilled water was circulated and temperature controlled by a Haake Model E-52 Heater/Circulator installed in a Varian Model 4000 constant temperature bath chamber;

(2) The variable wavelength ultraviolet detector was also thermostatted at 25^{o}C for greater baseline stability at high detector sensitivities;

(3) The electrometer on the detector was set at 0.5 absorbance units full scale and attenuation, 1;

(4) Injection volume was fixed at 10 µl (calibrated sample loop);

(5) The column was conditioned prior to usage to remove interfering polar aromatic impurities at a flow rate of 2.0 ml/min for four hours with isopropyl alcohol and reconditioned for another four hours at the same flow rate with the mobile phase composition cited above for the analysis (column outlet unattached).

(6) A Brownlee Labs guard column system was installed between the injector and the column consisting of a high performance disposable cartridge and a reusable holder to filter out harmful particles and irreversibly adsorbed materials from the sample, along with dust, teflon fragments from the injector valve, water, etc.

One measure of column efficiency is its number of effective theoretical plates, a concept derived from distillation theory. The greater the number of plates, the better apt the column to resolve components of similar polarity. The equation used for measuring the column's effective theoretical plates (n') was:

$$n' = 5.545 \left[\frac{(t_{R'})}{W_{0.5h}} \right]^2$$

a) where $t_{R'}$ = the adjusted retention time, i.e. absolute retention time minus column dead volume time (i.e. unretained toluene here);

b) and $W_{0.5h}$ = peak width, i.e. width of peak measured at half the peak's height.

The sample analyzed to determine column efficiency was a ten microliter injection of toluene and MDA dissolved in hexane to 100 ppm each and run at ambient conditions in the above mobile phase at the above flow rate (Figure 2).

From Figure 2,

$$n' = 5.545 \left[\frac{(11.2 \text{ cm} - 2.7 \text{ cm})}{(1.2 \text{ cm})} \right]^2$$

The effective theoretical plates were determined from this run to be approximately 275 for MDA.

c. Concentration Step

Two hundred milliliter samples of JP-4 containing neither deactivator nor internal standard were doped with MDA in the range of 0-6.0 ppm, the upper limit conforming with MIL-T-5624L. In addition, an internal standard, acetanilide (ACE) was added to each sample to a concentration of 5.0 ppm. The fuel samples were then evaporated at about 218^{o}C under a gentle stream of nitrogen (i.e. 100 ml/min). The fuel residue, approximately 5 ml, was transferred via a Pasteur pipette to a 10 ml volumetric flask and diluted with methylene chloride to the mark. In this manner, a scale up factor of 20 in concentration was realized for both the MDA and the ACE.

In summary, these synthetically prepared JP-4 fuel samples, after the 20-fold concentration increase, should theoretically range from 0.0 to 120.0 ppm for MDA and be 100.0 ppm for ACE. Subsequent calibration curves were generated using standard solutions that simulated these final concentrations for MDA and ACE.

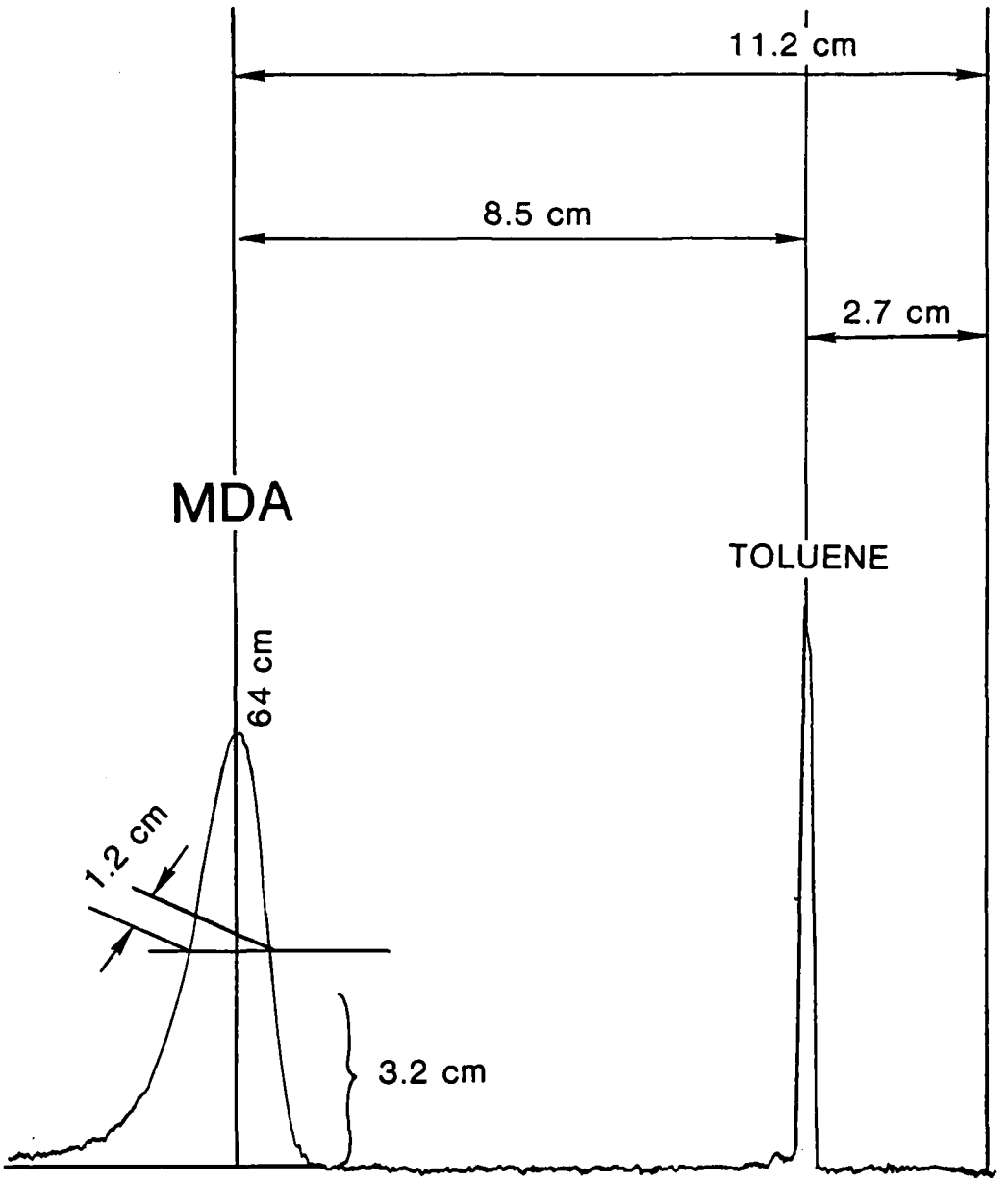


Figure 2. Effective Theoretical Plates of Column (in Analysis Mode)

2. QUALITATIVE IDENTIFICATION METHODS

a. Relative Retention Time

(1) Background

The chromatographic behavior of a component of interest can provide information on its identity. Simply matching absolute retention times of the metal deactivator concentrated from an actual fuel sample with that of the pure standard is a good indication of its identity. The absolute retention times of a particular component or peak is the time from injection to the peak maximum, as displayed on the recorder or logged by an integrator. The same compound on the same column under exactly the same conditions should have the same absolute retention time. However, slight deviations in any of the operating conditions or for that matter, the quality or state of the column, can change this absolute retention time. Also, it is possible for different compounds to exhibit identical retention times.

For these reasons, the determination of the retention time of the MDA relative to a chemically similar reference compound was preferred since it is far more reliable and repeatable from day-to-day, as will be demonstrated in this report. Whatever alters the retention time of the reference component will also affect other like components in a similar manner. Values for MDA's relative retention time are dependent only upon temperature and composition of the column and the eluent. If these are kept constant, so are the relative retention times. There are, however, several requirements for the successful employment of an internal reference compound:

(a) It must be completely resolved from the component of interest;

(b) It must be similar in concentration to the peak of interest;

(c) It must not react or decompose in the chromatographic system;

(d) It must elute near the peak of interest.

After considerable trial and error, a suitable reference standard was found in research grade acetanilide (ACE), purchased from Coleman Instruments, Division of Perkin-Elmer (Figure 3).

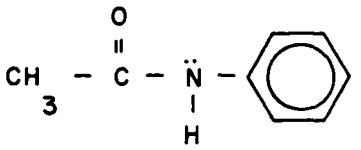


Figure 3. ACETANILIDE (ACE)

(2) Calibration Standards Data

Table 1 is a compendium of relative retention times derived from calibration blends containing MDA and ACE (see Section 3. "Quantitative Determination" for experimental details).

The equation used for measuring MDA's relative retention time was:

$$RRT_{MDA} = \frac{t_{R'\ MDA}}{t_{R'\ ACE}}$$

where $t_{R'}$ = the adjusted retention time, i.e. absolute retention time minus column dead time (for unretained toluene).

All sample preparations were chromatographed five times over a three day period. The relative standard deviation and 95% confidence limits are also displayed in Table 1. Table 2 lists the relative retention time data for the other accepted metal deactivator, N,N'-disalicylidene-1,2-cyclohexanediamine. Obviously, this column flowing with this mobile phase could resolve the two metal deactivators in the highly unlikely event that they were present in the same fuel sample.

TABLE 1

RELATIVE RETENTION TIME DATA*: N,N'
DISALICYLIDENE-1,2-PROPANE DIAMINE

$t_{R'}$ MDA	$t_{R'}$ ACE	RRT MDA
705	1169	0.5236
697	1156	0.5224
703	1163	0.5248
696	1154	0.5224
700	1160	0.5233
707	1171	0.5246
704	1165	0.5247
706	1170	0.5241
705	1168	0.5242
703	1165	0.5237
707	1174	0.5230
705	1170	0.5231
703	1166	0.5232
700	1158	0.5244
701	1158	0.5244
706	1169	0.5246
700	1160	0.5233
700	1161	0.5228
697	1154	0.5235
698	1156	0.5234
695	1152	0.5225
698	1154	0.5245
700	1160	0.5233
696	1150	0.5246
697	1154	0.5235
699	1156	0.5245
698	1155	0.5240
700	1159	0.5239
697	1155	0.5229
700	1158	0.5244
705	1168	0.5242
704	1169	0.5226
700	1160	0.5233
701	1160	0.5244
699	1159	0.5228
		$\overline{RRT}_{MDA}$ = 0.5237

*Statistical Information: standard deviation, σ , = 0.0007
relative standard deviation = 0.14%

where 95% confidence limit = $\frac{\sigma \cdot t}{(n)}$ 1/2

95% Confidence Limits=+/- 0.0003 given that n=35 runs and t=2.032 ("t"-Table)

TABLE 2

RELATIVE RETENTION TIME DATA*: N,N'-DISALICYLIDENE-1,2-CYCLOHEXANE DIAMINE

$t_{R'}$ MDA	$t_{R'}$ ACE	RRT MDA'
340	1195	0.2845
340	1190	0.2857
340	1195	0.2846
340	1195	0.2845
340	1190	0.2857
340	1185	0.2869
345	1196	0.2885
340	1185	0.2869
342	1188	0.2879
343	1193	0.2849
		$\overline{RRT}_{MDA'}$ = 0.2860

*Statistical Information: standard deviation, σ, = 0.0015
relative standard deviation = 0.51%

where 95% confidence limit = $\frac{\sigma \cdot t}{(n)}1/2$

95% Confidence Limits=+/- 0.0010 given that n=10 runs and t=2.2622 ("t"-Table)

b. Absorbance Ratioing

(1) Background

Although the relative retention time data exhibited excellent repeatability, RRT_{MDA} is not sufficient to positively identify particular peaks, since the data is characteristic, but not definitive. To significantly increase the validity of the qualitative assignment of the metal deactivator peak, absorbance ratioing with a variable wavelength spectrophotometric HPLC detector was introduced.

Absorbance ratioing can be used to significantly improve the component assignment reliability (Reference 3-6). If ratios of both standards and sample components match, purity and identity can be confirmed. If absorbance ratios fail to match, it is highly probable that the compound has been improperly identified or that the component of interest is unresolved from a contaminant. This latter case also results in erroneous quantitative information, thus the absorbance ratioing method can be viewed as essential in obtaining accurate quantitative analyses.

An ultraviolet spectrum of a component in a complex mixture is a good "fingerprint" of that compound but taken alone is difficult to interpret accurately. More precise information can be gained from absorbance ratios at several different wavelengths. These ratios, when obtained under standard conditions, are absolutely specific for a given compound and can be used to identify it and determine its purity. By stopping the column flow while the compound of interest resides in the detector cell, the concentration of the compound remains constant, and if Beer's Law is followed, a linear relationship exists between absorbance and concentration. Absorbance ratios, then, are actually ratios of extinction coefficients (Reference 7). The Varian HPLC system previously described is capable of stopflow operation. Flow is halted simply by closing a valve located near the column inlet. This action maintains the

pump's original operating pressure, but isolates the column. The procedure is relatively simple for determining absorbance ratios:

(a) As the peak elutes, it is isolated or "trapped" in the flowcell of the spectrophotometer by stopping the flow,

(b) The computing integrator's microvolt level is recorded at a given wavelength,

(c) The wavelength selector is carefully reset at another wavelength and the level recorded,

(d) This process is repeated enough times to accumulate data for five ratios relative to the generally used 254 nm,

(e) The original wavelength is reset and the peak allowed to elute,

(f) After the baseline is re-established, background microvolt levels are obtained at each wavelength (to be subtracted from the corresponding sample readings).

Wavelengths were selected to include: a general wavelength, i.e. 254 nm, where many aromatic compounds have some absorption, a lower wavelength, i.e. 244 nm, where most aromatic compounds have stronger absorption, and several other wavelengths up to nearly 300 nm. It is crucial to determine the ratios for three or more wavelengths, because it is quite possible for the ratios to match for any two wavelengths. However, it is highly unlikely for ratios to match at three or more well-resolved wavelengths.

(2) Calibration Standards Data

Table 3 is a compilation of runs of a 100 ppm standard solution of MDA , and Table 4 is a statistical analysis of that data. The flow was randomly stopped at various sites on the leading, crest, and tailing edge of peaks. Note, however, that the relative standard deviations of the ratios were less than one percent, making

TABLE 3

ABSORBANCE RATIOS FOR MDA

λ	Level	Level'	R(λ/λ 254)	Level	Level'	R(λ/λ 254)	Level	Level'	R(λ/λ 254)	Level	Level'	R(λ/λ 254)
	Run 1			Run 2			Run 3			Run 4		
254	2750	1750	-----	2320	1320	-----	3355	2355	-----	3450	2450	-----
244	5180	2030	1.160	4682	1532	1.161	5875	2725	1.157	5970	2838	1.158
264	1838	1818	1.039	1380	1360	1.030	2464	2444	1.038	2558	2538	1.036
274	1350	1520	0.869	968	1138	0.862	1890	2060	0.875	1970	2140	0.873
284	2320	1290	0.737	2010	980	0.742	2768	1738	0.738	2830	1800	0.735
294	2489	1389	0.794	2130	1030	0.780	2913	1813	0.770	3006	1906	0.778
	Run 5			Run 6			Run 7			Run 8		
254	2280	1280	-----	2320	1320	-----	2315	1315	-----	2320	1320	-----
244	4630	1480	1.156	4675	1525	1.155	4674	1524	1.159	4685	1535	1.163
264	1350	1330	1.039	1380	1360	1.030	1380	1360	1.034	1380	1360	1.030
274	933	1103	0.862	973	1143	0.826	970	1140	0.867	979	1149	0.871
284	1980	950	0.742	2000	970	0.735	2010	980	0.745	2000	970	0.735
294	2115	1015	0.793	2145	1045	0.792	2130	1030	0.783	2150	1050	0.795
	Run 9			Run 10			Run 11			Run 12		
254	3230	2230	-----	2485	1485	-----	3060	2060	-----	3230	2230	-----
244	5732	2582	1.158	4878	1728	1.164	5540	2390	1.160	5730	2580	1.157
264	2333	2313	1.037	1565	1545	1.040	2158	2138	1.038	2330	2310	1.036
274	1732	1902	0.853	1112	1282	0.863	1630	1800	0.874	1780	1950	0.874
284	2650	1630	0.731	2132	1102	0.742	2565	1535	0.745	2670	1640	0.735
294	2850	1750	0.785	2240	1140	0.762	2694	1594	0.774	2848	1748	0.784

Note: Background Microvolt Levels 254=level 1000; 244=level 3150; 264=level 20; 274=level -170; 284=level 1030; 294=level 1100.

TABLE 4

STATISTICAL ANALYSIS OF ABSORBANCE RATIOS FOR MDA

n-values	$\bar{R}$ ($\lambda_n / \lambda_{254}$)	Standard Deviation	Relative Standard Deviation	95% Confidence Limit (+/-)
244	1.159	0.003	0.24%	0.002
264	1.036	0.004	0.36%	0.003
274	0.867	0.007	0.76%	0.003
284	0.739	0.005	0.62%	0.003
294	0.783	0.010	1.32%	0.006

absorbance ratioing an acceptable criterion for the qualitative identification.

c. Jet Fuels Analysis Data

Having measured peak ratios for pure MDA at 100 ppm level, the concentrates of six jet fuel samples were examined for MDA presence via the above procedure (see subsection 3 "Quantitative Determination" for experimental details). Variations of more than about 2% relative standard deviations in peak absorbance ratios compared with the above standard solution of MDA is supposed to be an indication that a component is not MDA (Reference 3). However, Table 5 shows the excellent agreement of ratios comparing MDA in the standards and the fuel samples. The averaged absorbance ratios for each wavelength are listed next to the corresponding ratios for the MDA peaks tentatively identified by relative retention times in the six fuel samples. Of all the fuel samples analyzed, only one exceeded the relative standard deviation limits of +/- 2.0% for the MDA standard average given in Table 1.

3. QUANTITATIVE DETERMINATION

a. Background

In any chromatographic analysis, the separation between the component(s) of interest and interfering compounds must be optimized before quantitation can be properly addressed. Previous work and preliminary experiments with mobile phase compositions of varying solvent polarity attempted to elute the MDA in a reasonable time while ensuring baseline resolution from the ACE as well as from the tailing peak of the residual aromatic constituents of the evaporated fuel sample. Peak tailing was minimized by spiking the methylene chloride mobile phase with glacial acetic acid. Blank runs of sample solvent as well as JP-4 concentrate (without additives) indicated that potentially interfering peaks that might co-elute with either the MDA or the ACE were absent.

TABLE 5

MDA ABSORBANCE RATIOS: FUEL SAMPLE V.S. STANDARD SOLUTION

λ_n	Standard's $R(\lambda_n / \lambda_{254})$	Found* Values of $R(\lambda_n / \lambda_{254})$ For Fuel Sample Codes:					
		MDA-1 RD(%)	MDA-2 RD(%)	MDA-3 RD(%)	MDA-4 RD(%)	MDA-5 RD(%)	MDA-6 RD(%)
244	1.159	1.178 +1.64	1.169 +0.86	1.165 +0.52	1.158 -0.09	1.157 -0.17	1.156 -0.26
264	1.036	1.054 +1.74	1.047 +1.06	1.041 +0.48	1.035 -0.10	1.034 -0.19	1.032 -0.39
274	0.867	0.883 +1.85	0.878 +1.27	0.876 +1.03	0.867 -----	0.866 -0.12	0.865 -0.23
284	0.739	0.755 +2.17	0.754 +1.49	0.746 +0.95	0.738 -0.14	0.738 -0.14	0.737 -0.27
294	0.783	0.799 +2.04	0.795 +1.53	0.787 +0.51	0.782 -0.13	0.781 -0.26	0.780 -0.38

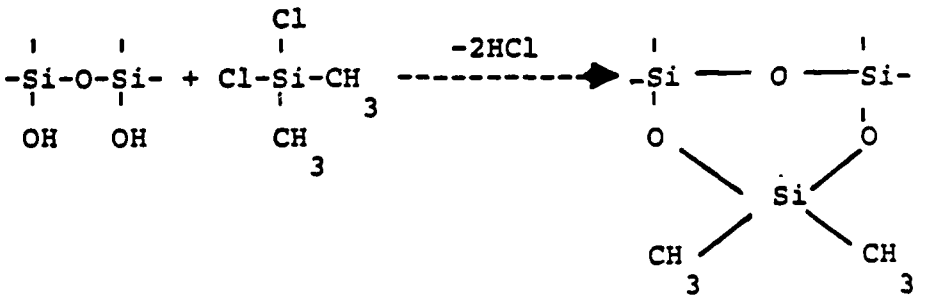
*Average of three determinations at crest of suspected MDA peak

Note: RD(%) = Relative Deviation in Percent

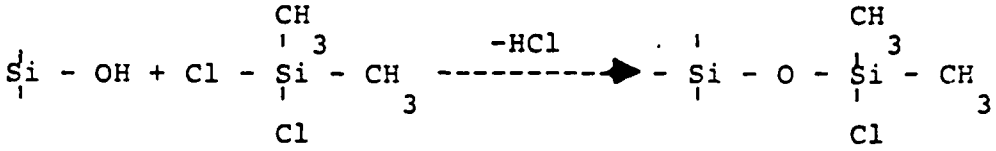
In developing any multi-step analytical method, analyte losses must be evaluated and minimized after each critical step. It was theorized that since the polar characteristics of the MDA and ACE were similar on this column and with this mobile phase under the above operating conditions (see the previous subsection, "Qualitative Identification Methods"), and since both components were relatively involatile, losses in recovering the MDA due to improper handling, precipitating out on the container walls, oxidation, etc. would be comparable. Each fuel sample was doped with the same amount of ACE and upon evaporation, the recovered ACE used as the indicator of the total recovery of the MDA. The found amount of MDA was corrected for recovery losses by a factor that was determined by the found amount of ACE.

Losses due to sampling were minimized. All MDA and ACE calibration solutions were stored in a refrigerator maintained at $10^{o}C$ and chromatographed within three days of their preparation. No degradation of the MDA or of the ACE was noted as a function of time. All glass containers were thoroughly washed with ALCONOX, and rinsed with distilled water, followed by HPLC grade solvents. They were then oven dried and placed in a dessicator for cooling and storage until use. The bottles were subsequently silanized with "Glas-TREET" (Alltech Associates).

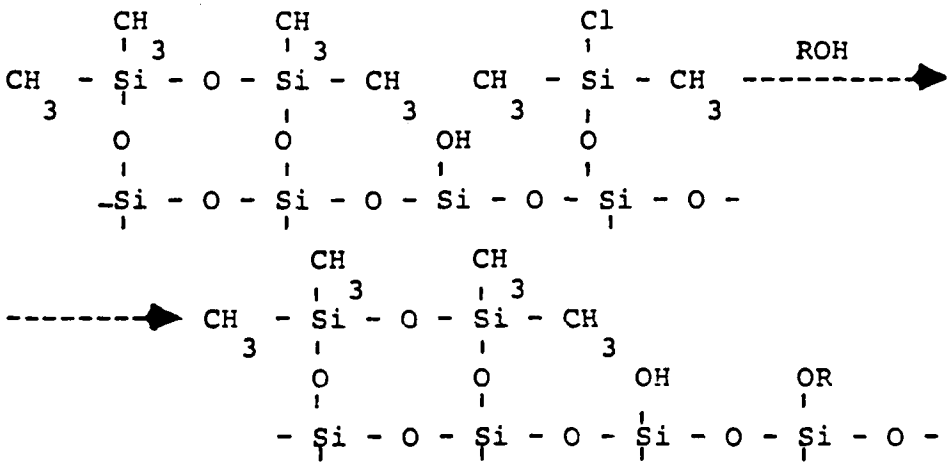
The active ingredient, dimethyldichlorosilane, is the most efficient silylation agent used in the surface deactivation of diatomaceous earth materials used as stationary supports (Reference 9). The reaction with vicinal surface hydroxy groups of the glass proceeds rapidly:



However, reactive chlorines may remain in the surface layer if two vicinal groups are not available for reaction (Reference 9):



A methanol wash removes HCl from the surface and also hydrolyzes the remaining active chlorines. The following mechanism may occur in the alcohol treatment (Reference 9):



Sample introduction errors into the high pressure system were reduced significantly by a fixed volume loop injector. The sampling valve was air-actuated, controlled by the AutoLab System IV Computing Integrator. The valve injection system demonstrated superior precision ($\leq$ 1%) when compared to hand-held syringe injections (2-3%). The principal advantages of a loop injector are:

(1) The volume injected is far more repeatable since a fixed volume loop has a constant volume and is flushed with a relatively large sample volume in the filling mode;

(2) The rate of sample injection is not a variable;

(3) Since the operator is removed from the sequence, technique differences are avoided;

(4) The valve is automated and injection can be initiated at precise intervals, thus maintaining constant state of column equilibration.

Detection and quantitation of the separated MDA was achieved with a variable ultraviolet detector. The sample concentrates were diluted in the non-ultraviolet absorbing mobile phase to avoid over-saturating the detector at high absorbances. Calibration curves (Figures 4 and 5) generated for MDA and ACE confirm the excellent linearity in the ppm range of interest to this analysis. Peak integration, as performed by the AutoLab Computing Integrator, was merely the accumulation of microvolt signals from the electrometer of the detector reported as area counts.

Calculations to obtain the actual concentrations of MDA and ACE in a fuel concentrate blend were simplified. Since injection volumes were so repeatable, the external standardization technique of quantitation could be employed. This method is the direct comparison of peak areas of components in an unknown sample with the corresponding peak areas in standard solutions. A calibration curve was generated for each component from a series of known concentrations and the corresponding peak area responses. In each case, a first order least squares curve fit provided the equation of the line. A fuel sample was evaporated as described above with exactly the same volume injected. The MDA and the ACE amounts present were calculated from the respective peak areas substituted into the equation for the calibration curve for that component.

b. Calibration Standards Data

The JP-4 base stock was analyzed for any trace MDA present before preparation of the calibration standards. The actual fuel concentrates (described in subsection 2) were filtered through an unsilanized 0.45 micron syringe filtering assembly (Millipore Part No. XX3001200) prior to introduction into the HPLC column.

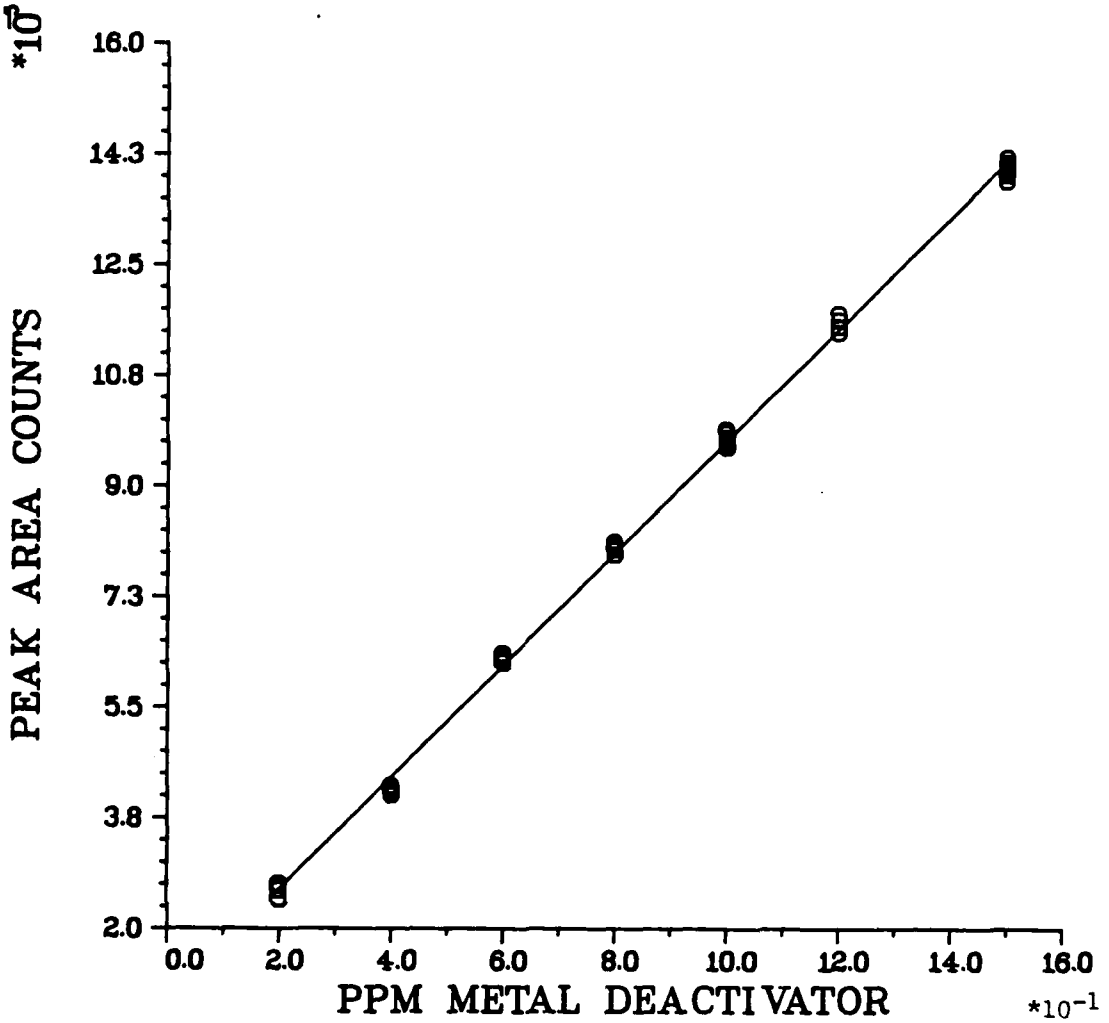


Figure 4. External Calibration Curve for Metal Deactivator

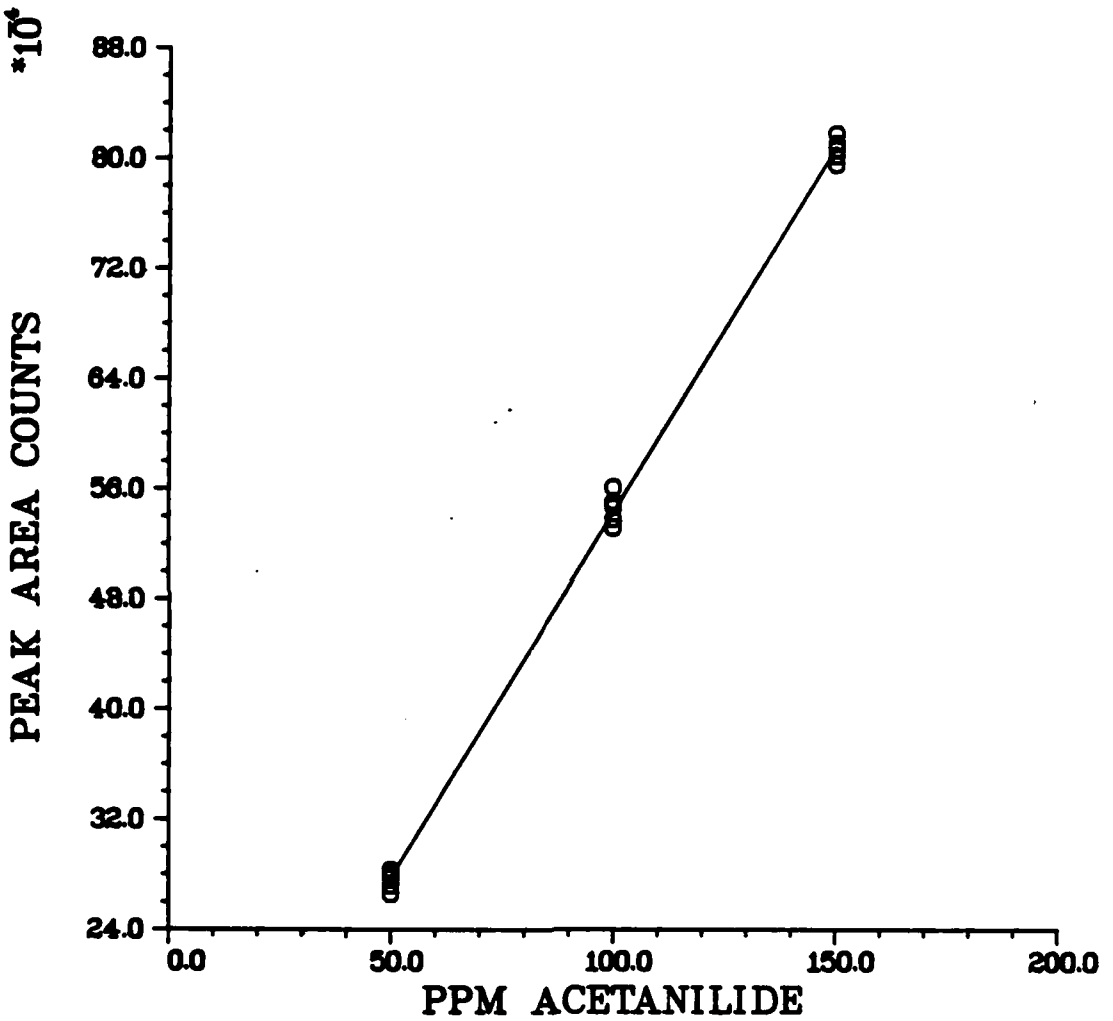


Figure 5. External Calibration Curve for Acetanilide

A series of blends containing MDA and ACE were prepared, chromatographed, and peak areas calculated. Figures 4 and 5 are plots of the results. Statistical analysis of this data by least squares curve fitting gave correlation coefficients that approached unity (see Table 6). With the linear equation for the unique calibration curve of each component, the unknown fuel concentrates could then be analyzed for MDA and ACE by substitution of the peak area counts from that run into the appropriate calibration curve equation.

These equations were then rearranged so that the amount of MDA additives in an unknown sample, i.e. "X," could be predicted via interpolation given the appropriate area counts from its HPLC run, i.e. "Y." The "standard error of estimate" cited in Table 6 has properties analogous to that of the standard deviation about a mean value. Lines constructed parallel to each regression line, of "X" values (ppm of component) for given "Y" values (peak area counts), at respective vertical distance of one, two, and three standard deviations from it, include 68%, 95%, and 99.7% of the data points. Small errors (relatively) of estimate indicate precise, repeatable results.

Given the high degree of correlation, a statistical analysis was performed on the calibration curves to predict the uncertainty in a value of "X," i.e. ppm of component, associated with an observation or series of observations on the dependent variable "Y," i.e. area counts from integrator. The attendant equations employed and a description of how they were obtained are included in the Appendix. The 95% confidence intervals for the true value of the predicted ppm amount of MDA and ACE at typical concentration levels are included in Table 7, as described in the Appendix.

This table, in effect, states that if the MDA calibration curve predicts that a given unknown solution has 150 ppm of MDA, there is a 95% probability that the true value lies between +0.44 ppm and -0.44 ppm from that predicted value.

TABLE 6

STATISTICAL PARAMETERS OF CALIBRATION CURVE PLOTS FOR MDA AND ACE

Component	Equation of Line for each Calibration Curve*	Correlation Coefficient	Standard Error of Estimate
MDA	$Y = 8.898 \times 10^{4} X + 8.477 \times 10^{4}$	0.9957	3.549×10^{4}
ACE	$Y = 5.306 \times 10^{3} X + 1.202 \times 10^{4}$	0.9992	9.009×10^{3}

*Note: Y = HPLC peak area counts
X = component's known ppm

c. Jet Fuel Analysis Data

(1) Background

As noted previously, two hundred milliliter samples of a JP-4 jet fuel were doped with MDA to meet the Air Force fuel specification limits for that additive. ACE also had been added to eventually yield, upon evaporation, a level of 100 ppm in the fuel concentrate. After the evaporation step, the MDA concentrations were then comparable to those of the original MDA calibration curve. All the fuel samples were evaporated down and the fuel residue transferred to a 10 ml volumetric flask and diluted to the mark with methylene chloride. Recall that the ACE had been added prior to this 20-fold concentration step to serve not only as a recovery standard but also as a relative retention time standard. The concentrates were then chromatographed.. The results are shown in Figure 6, the theoretical compositions in Table 8.

(2) ACE Recovery Results

The equation for the linear least squares line (see Table 6) for ACE was rearranged so that its amount remaining in the fuel concentrate, i.e. "X" could be predicted from the area counts of that sample's HPLC run, i.e. "Y." Table 9 displays the average results of the fuel samples containing both MDA and ACE. The ACE percentage recovered was calculated by dividing the theoretical amount of ACE present, i.e. originally added to the sample, into the found amount of ACE predicted from the area counts of the HPLC run and the ACE calibration curve, and multiplying that quotient by 100. The results show a reasonably good recovery level of the ACE with good repeatability of its determination from sample to sample. A similar table was generated for the MDA results to check that approximately the same relative amounts of MDA were being recovered as well. Table 10 lists those results. The recovery of the MDA was not as high as for the ACE but was on a level adequately comparable to that for ACE.

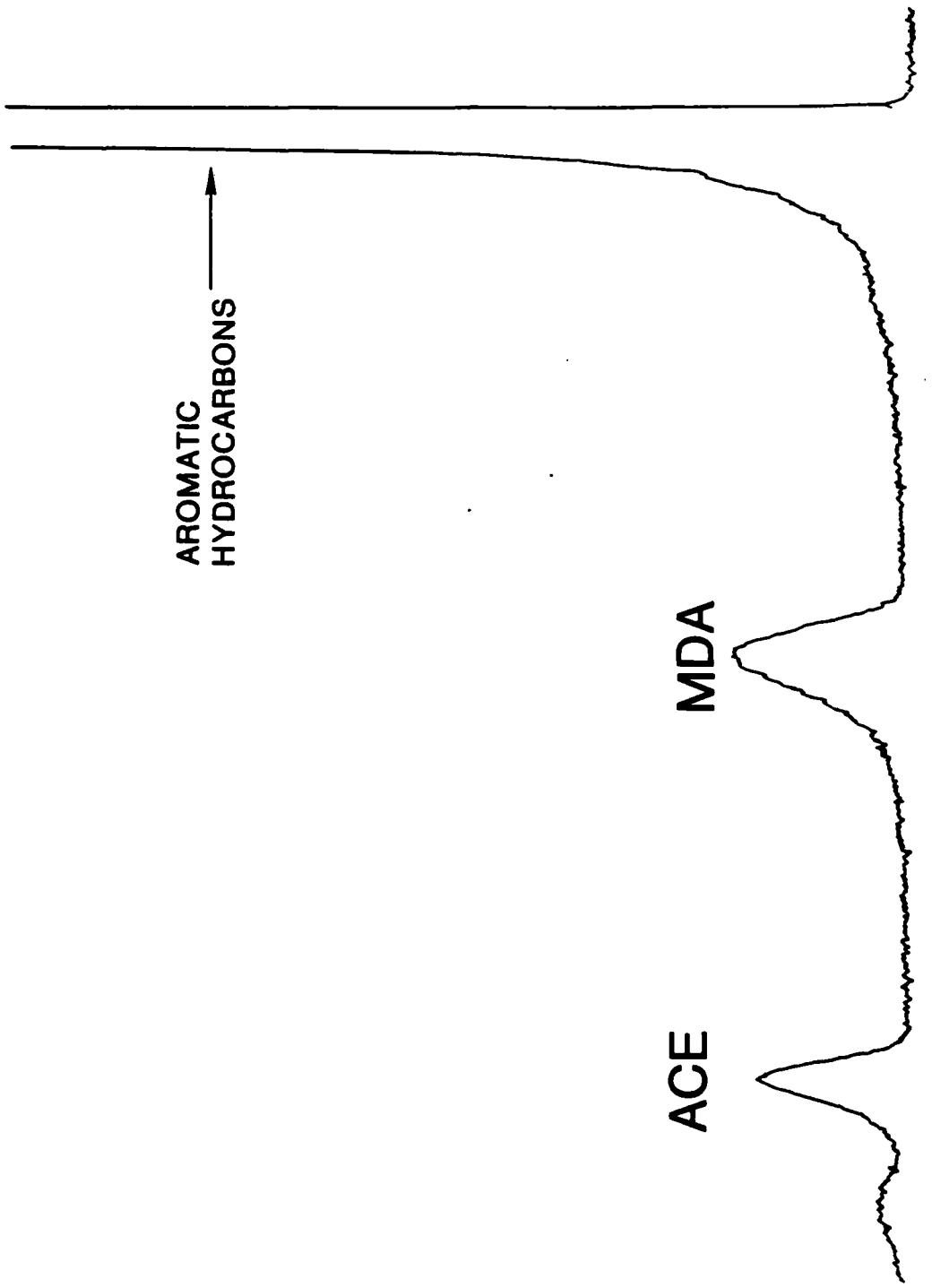


Figure 6. HPLC Run on JP-4 Fuel Concentrate Containing Metal Deactivator and Recovery Standard (See text for operating conditions)

TABLE 7

UNCERTAINTY IN PPM VALUES AT DIFFERENT
ADDITIVE LEVELS AT 95% CONFIDENCE INTERVAL

Additive	Concentration	Associated Uncertainty
MDA	20 ppm	+/- 0.49 ppm
	150 ppm	+/- 0.44 ppm
ACE	100 ppm	+/- 0.11 ppm

TABLE 8

THEORETICAL COMPOSITIONS OF JET FUEL CONCENTRATES

FUEL SAMPLE CODES

sample ID	theor. amt. MDA (ppm)	theor. amt. ACE (ppm)
blank	0	0
mda-0	0	100
mda-1	20	100
mda-2	40	100
mda-3	60	100
mda-4	80	100
mda-5	100	100
mda-6	120	100

TABLE 9

RECOVERY PERCENTAGE FOR CONCENTRATION STEP (ACE)*

Sample Code	Theoretical Amount of ACE (ppm)	Found Amount of ACE (ppm)	Recovery Percentage (%)
MDA-1	100.0	95.5	95.5
MDA-2	100.0	97.0	97.0
MDA-3	100.0	96.7	96.7
MDA-4	100.0	96.1	96.1
MDA-5	100.0	97.3	97.3
MDA-6	100.0	97.5	97.5
MDA-0	100.0	97.8	97.8
BLANK	0.0	0.0	0.0

Mean Recovery = 96.8

*Statistical Information: standard deviation, σ , = 0.8%
relative standard deviation = 0.8%

where 95% confidence limit = $\frac{\sigma \cdot t}{(n)}1/2$

95% Confidence Limits=+/- 0.9% given that n=7 runs and t=2.9687 ("t"-Table)

TABLE 10

RECOVERY PERCENTAGE FOR CONCENTRATION STEP (MDA)*

Sample Code	Theoretical Amount of MDA (ppm)	Found Amount of MDA (ppm)	Recovery Percentage (%)
MDA-1	20.0	18.8	94.0
MDA-2	40.6	38.2	95.5
MDA-3	60.0	58.0	96.7
MDA-4	80.0	76.2	95.3
MDA-5	100.0	96.3	96.3
MDA-6	120.0	116.5	97.1
MDA-0	0.0	0.0	0.0
BLANK	0.0	0.0	0.0
			Mean Recovery = 95.8

*Statistical Information: standard deviation, σ, = 1.1%
relative standard deviation = 1.2%

where 95% confidence limit = $\frac{\sigma \cdot t}{(n)}$ 1/2

95% Confidence Limits=+/- 1.5% given that n=6 runs and t=3.1634 ("t"-Table)

(3) Final MDA Determinations

The analytical scheme requires the found amount of MDA be corrected for recovery losses as indicated by the found amount of the recovery standard, ACE. Table 11 reports the corrections in the results of the previously analyzed samples listed in Tables 9 and 10. The corrected amount of MDA in a particular fuel sample was calculated by multiplying the uncorrected amount of MDA found in that sample by the ratio of the amount of ACE added to that sample divided by the found amount of ACE for that sample.

TABLE 11

MDA DETERMINATIONS AFTER RECOVERY CORRECTIONS

Sample Code	Theoretical Amount MDA (PPM) in Concentrate	Found Amounts MDA (PPM) In Concentrate	
		Uncorrected for ACE Recovery	Corrected For ACE Recovery
MDA-1	20.0	18.8	19.7
MDA-2	40.0	38.2	39.4
MDA-3	60.0	58.0	60.0
MDA-4	80.0	76.2	79.3
MDA-5	100.0	96.3	99.1
MDA-6	120.0	116.5	119.5

SECTION IV

SUMMARY AND SUGGESTIONS

A successful analytical method has been developed that qualitatively identifies and quantitatively determines a metal deactivator additive used in Air Force JP-4 jet fuel. Relative retention time calculations, together with ultraviolet absorbance ratios at various wavelengths, were highly specific in assigning the tentative identification of the additive. A recovery standard that doubled as the internal reference standard for relative retention time measurements was crucial in correcting the found metal deactivator ppm level for losses in sample transfer, sample concentration, dilution, etc. A precision statement, reflecting the quantitative reliability of the method, cited +/- 0.5 ppm as the 95% confidence intervals for the true value of the predicted amount from the calibration curve for the metal deactivator in the 20-150 ppm range, after the 20-fold fuel concentration step.

By combining an optimized mobile phase composition with the ultraviolet detector, potential interferences were effectively screened out from the JP-4 matrix: fuel residues, e.g. saturated paraffins, alkyl benzenes, alkyl naphthalenes, and polynuclear aromatics; impurities, e.g. trace levels of pyridines and quinolines, plasticizers, etc.; and additives, e.g. alkyl phenols, fatty acids, a fatty alcohol, etc. Thus the separation scheme is of universal applicability for all specification JP-4 jet fuels regardless of the additive package used or the trace contaminants normally encountered. The versatility of the analytical method was further exemplified by its correct qualitative analysis of synthetic blends that contained either formulation of the two Air Force accepted metal deactivators.

Future work on this particular additive should include the monitoring of the visible radiation wavelengths via the ultraviolet-visible spectrophotometer. The analytical scheme could then be even more selective and definitive for the metal deactivator or, for that matter, for other JP-4 distillate fuel additives that are colored, since the vast majority of all fuel components, including additives and impurities, show no response to radiation in the visible wavelengths.

APPENDIX

SUBJECT: Predicting the uncertainty in a value of X (part per million of a given component) associated with an observation or series of observations, on the dependent variable Y (area counts as determined by the HPLC's Integrator).

Using the output from an available subroutine called "STATIST," and using the equation on pp. 231-232 of "Statistical Analysis in Chemistry and the Chemical Industry" by C. A. Bennet and N. L. Franklin, J. Wiley & Sons, 1954; the following parameters are required:

b = the slope of the original calibration curve

σ^2 = square of the standard error of estimate

Y_o = the mean of the m new observations, (where m = # of new observations)

$\bar{y}$ = the mean of the original area percentages used in developing the calibration curve

X_i = the original values of volume percentages used to derive the calibration curve

n = the number of points (pairs) in the original calibration curve

t-value = value of student's t from a t-Table (with m-1 degrees of freedom)

LET X_o = ppm MDA Level of Interest

Examples:

CASE I (MDA Calibration Curve)

For X_o = 20 ppm MDA (Final Concentrated Solution)

Then: $b = 8.89796 \times 10^{+4}$

From "STATIST": $\sigma^2 = 12.60144 \times 10^{+8}$

$n = 40$

And: $Y_o = 2.58682 \times 10^{+5}$ (with m = 5)

$\bar{Y} = 8.16628 \times 10^{+5}$

Using equation (g) on page 232:

$$\text{Var}(X_o) \sim \frac{\sigma^2}{b^2}\left(\frac{1}{m} + \frac{1}{n} + \frac{(Y_o - \bar{y})^2}{b^2 \Sigma_i (X_i - \bar{X})^2}\right) \qquad (1)$$

The following identity can be substituted:

$$\sigma(X_i - \bar{X})^2 = \Sigma(X_i)^2 - \frac{(\Sigma X_i)^2}{n}$$

In this case: $\sigma(x_i - \bar{X})^2 = 716.975$ and;

$$(Y_o - \bar{y})^2 = 31.1303 \times 10^{10}$$

Substituting into equation (1) :

$$\text{Var}(X_o) = \frac{(12.60144 \times 10^{+8})}{(79.17366 \times 10^{+8})}\left(\frac{1}{5} + \frac{1}{40}\right) + \frac{(31.13032 \times 10^{10})}{(79.17366 \times 10^{8})(716.975)}$$

$$\text{Var}\ (X_o) = 0.04454$$

$$S(X_o) = \text{Var}\ (X_o)^{1/2}$$

$$S(X_o) = 0.2110$$

The 100 $(1 - \alpha)$% confidence limits for X_o are:

$$X_o - t(n-2,\ 1 - \tfrac{\alpha}{2})\ S(X_o) < \bar{x} + \frac{Y_o - \bar{y}}{\beta} < X_o + t(n-2,\ 1 - \tfrac{\alpha}{2})\ S(X_o) \qquad (2)$$

This equation simply states that the 95% confidence interval for the true value of X_o

where the true value of X_o is:

$$\text{True value of } X_o = \bar{x} + \frac{Y_o - \bar{y}}{\beta}$$

and β = true slope

lies between $X_o - t(n-2,\ 1 - \frac{\alpha}{2})\ S(X_o)$

and $X_o - t(n-2,\ 1 - \frac{\alpha}{2})\ S(X_o)$

If we select $\alpha = 0.05$ (i.e. the 100 $(1 - 0.05)$% or 95% confidence level) then from the t-Tables:

$$t(40 - 2),\ 1 - \frac{0.05}{2}) = t(38,\ 0.025) = 2.335$$

$$X_o - (2.335)\ (0.2110) < \text{True value of } X_o < X_o + (2.335)(0.2110)$$

Therefore for X_o = 20 ppm MDA, 19.51 ppm < True value of

$$X_o < 20.49 \text{ ppm}$$

This confidence internal about X_o at this ppm level represents about ± 2.22% relative.

Case II (MDA Calibration Curve)

For X_o = 150 ppm MDA (final concentrated solution)

Application of the above equations yields:

149.56 ppm < True value of X_o < 150.44 ppm

or about ± 0.30% relative

Note that the maximum allowed concentration of metal deactivator (5.8 ppm) would yield, after the fuel concentration steps, a final level of 116 ppm. It is reasonable to state that the 95% confidence limits at that concentration would be less than ± 0.45 ppm absolute!

CASE III (ACE Calibration Curve)

For X_o = 100 ppm ACE (final concentration solution)

Application of the above equation yields:

99.89 ppm < True value of X_o < 100.11 ppm or about ± 0.11% relative.

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